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CYTOGENETIC ANALYSIS OF TETRAPLOID WHEAT USING COMMON WHEAT ANEUPLOIDS

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Cytogenetic analysis of tetraploid wheat using common wheat aneuploids" submitted by Leonard Arnold Millis in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The use of monosomics to establish gene-chromosome associations in the hexaploid wheats suggests that certain crosses between tetraploid wheats and hexaploid wheat monosomics might be useful to establish gene-chromosome associations in the tetraploid wheats. Monosomics of lines I to XIV of hexaploid spring wheat varieties were crossed with a tetraploid winter wheat variety. A cytogenetic analysis of the F_1 was made in an attempt to determine which chromosome or chromosomes of the tetraploid variety carry genes affecting growth habit.

It was found that chromosome IX and possibly chromosome V of the tetraploid variety carry genes affecting growth habit.

The theoretical basis and the value of the method are discussed, and comparisons with another possible method of analysis of tetraploids, using common wheat aneuploids, are made.

ACKNOWLEDGEMENTS

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INTRODUCTION

Genetic analysis has been useful in establishing the number and allelic relationships of genes controlling certain characters in hexaploid and tetraploid wheats (review by Ausemus, et al., 1946). Attempts at conventional linkage analysis, however, have rarely been successful. Because much of the genetic material in polyploids is duplicated or triplicated, segregation at one locus may be obscured by segregation at other loci. Conventional linkage studies on polyploids are therefore usually inconclusive.

With the isolation of aneuploids in the hexaploid wheats (Sears, 1939, 1944, and unpublished), an alternative to conventional linkage methods became available. Monosomics and nullisomics were used to establish gene-chromosome associations and to determine the action of various genes in hexaploid wheats (review by Unrau, 1950). Kuspira and Unrau (1959) have discussed methods of F_1, F_2 and F_3 aneuploid analysis and their usefulness in establishing gene-chromosome associations. Substitution lines have also been utilized in the hexaploid wheats to establish gene-chromosome associations (Sears, et al., 1957; and Kuspira and Unrau, 1957).

Monosomics have proven useful in the analysis of Nicotiana tabacum, a tetraploid (review by Clausen and Cameron, 1944). Monosomic series are presently being developed in the tetraploid cotton Gossypium hirsutum (review by Brown and Endrizzi, 1964) and in Avena sativa, a hexaploid (McGinnis, 1962).

Although complete series of monosomics have been established in the hexaploid wheats, and a series of monosomics has been established in Nicotiana tabacum, no such series has been established in the tetraploid wheats. Since conventional methods of linkage analysis in the tetraploid wheats are generally unsuccessful, and since no monosomic series exist in these wheats, alternative methods of linkage analysis are desirable.

One method by which the location of certain genes might be determined in the tetraploid wheats is by the analysis of the F_1 of certain crosses between hexaploid monosomics and tetraploids. In this thesis the theoretical basis for such a method is discussed, the results of such crosses are reported, and the value of the method is assessed.

LITERATURE REVIEW

1. Cytogenetic studies in wheats and the basis for such studies

The term wheat refers to all species of the genus Triticum. The wheats consist of diploid, tetraploid, and hexaploid species with 14, 28, and 42 chromosomes respectively. On the basis that chromosome pairing during meiosis in hybrids reflects homology, the AA, AABB, and AABBDD genomes have been assigned to the diploid, tetraploid, and hexaploid wheats respectively.

Studies made by McFadden and Sears (1944) and Kihara (1944), as cited by Riley, Unrau, and Chapman (1958), and further work by Riley and Chapman (1960), provide strong cytological evidence that Aegilops squarrosa is the donor of the D genome of hexaploid wheat. Both cytological (Riley, Unrau, and Chapman, 1958) and morphological (Sakar and Stebbins, 1956) evidence suggest that Aegilops speltoides is the donor of the B genome of the polyploid wheats.

Intragenomic and intergenomic pairing during meiosis have been shown to be under genetic control (Riley, 1960), which suggests that the absence of intergenomic pairing does not necessarily imply that extreme or considerable genetic differences exist between the genomes. Moreover, radiation studies (Stadler, 1929), genetic studies (review by Gaines, 1927; and review by Ausemus, et al., 1946), studies of nullisomic-tetrasomic compensation (Sears, 1952), and cytological studies (Sears and Okamoto, 1958) indicate that tetraploid and hexaploid wheats possess considerable duplication of genetic material.

The duplication and possible triplication of genetic material has permitted the existence and establishment of chromosome deficient aneuploids in hexaploid wheat. Several monosomic plants were found among the aberrant offspring of a haploid of Triticum aestivum var. Chinese Spring pollinated by a diploid of the same variety (Sears, 1939). Nullisomics were obtained from self-fertilized monosomics. Other monosomics were obtained from a partially asynaptic nullisomic, so that all 21 lines of monosomics have been established in the variety Chinese Spring (Sears, 1939, 1944, and unpublished). Subsequently other workers have utilized the chromosome deficient series in Chinese Spring to establish similar series in other hexaploid varieties.

The establishment of chromosome deficient series is not limited to the hexaploid wheats. A complete series of monosomics has been established in Nicotiana tabacum (Clausen and Cameron, 1944), and at present similar series are being developed in Avena sativa (McGinnis, 1962) and in Gossypium hirsutum (Brown and Endrizzi, 1964).

Wheat chromosomes were numbered according to the order in which monosomics were obtained and such that those chromosomes homologous to the tetraploid wheats (A and B genomes) were numbered 1 to 14 and those of the D genome were numbered 15 to 21. Larson (1954), mainly on the assumption that only chromosomes of the B genome contribute to stem solidness, tentatively placed chromosomes II, III, IV, V, VI, VII, and XIV in the A genome, and chromosomes I, VIII, IX, X, XI, XII, and XIII in the B genome. More critical

cytological evidence, provided by Okamoto (1962), based on the pairing of telocentric chromosomes of a hexaploid (AABBDD) with an artificially synthesized tetraploid (AADD) has resulted in a reclassification of the chromosomes belonging to the A and B genomes.

As a result of the placement of the 21 chromosomes of hexaploid wheat into seven homoeologous groups on the basis of the ability of tetrasomes to compensate for nullisomes (Sears, 1952), and on the basis of preliminary results concerning the identification of chromosomes belonging to the A and B genomes (Okamoto, 1957), Sears (1958) proposed a more informative numbering system which places the chromosomes in their respective genomes and simultaneously indicates their respective homoeologies with chromosomes of the other genomes. In the new numbering system chromosomes II and XIII were tentatively placed in the A and B genomes respectively; however, the more complete results of Okamoto (1962) suggest that the reverse placement is probably correct. The old and the new numbering system, with the correction for chromosomes II and XIII, are presented in Table I.

Table I. The new and old numbers of the chromosomes of hexaploid wheat (after Okamoto, 1962).

Homoeologous group	Genome A		Genome B		Genome D	
	New	Old	New	Old	New	Old
1	1A	XIV	1B	I	1D	XVII
2	2A	XIII	2B	II	2D	XX
3	3A	XII	3B	III	3D	XVI
4	4A	IV	4B	VIII	4D	XV
5	5A	IX	5B	V	5D	XVIII
6	6A	VI	6B	X	6D	XIX
7	7A	XI	7B	VII	7D	XXI

Once obtained, monosomics tend to form fairly stable lines in the sense that self-fertilized monosomic plants will generally give rise to a fairly consistent proportion of monosomic offspring. Self-fertilized monosomics produce approximately 24 percent disomics, 73 percent monosomics, and 3 percent nullisomics (Sears, 1953).

Morrison (1953), by counting chromosomes in the dividing nuclei of pollen grains, and Morrison and Unrau (1952), by determining the frequency of micronuclei in pollen quartets, indicate that although the figures differ somewhat, depending on the chromosome concerned, approximately 75 percent of the pollen grains produced by a monosomic are chromosome-deficient. From the frequency of monosomics in the offspring of monosomics pollinated by disomics, Sears (1944) has estimated that approximately 75 percent of the

functional eggs are chromosome deficient. Tsunewaki and Heyne (1960), in similar studies, have found this figure to vary from 52 to 89 percent, depending on the chromosome concerned.

The high proportion of disomics produced from a self fertilized monosomic indicates that there is likely considerable certation among the pollen grains, with the 21-chromosome pollen being considerably more effective in fertilization than the 20-chromosome pollen.

Although monosomic plants may generally be considered to form stable lines, Person (1956) has shown that monosomic plants tend to be partially asynaptic and suggests that a monosomic line might become deficient for a chromosome other than the one for which it was originally deficient. Such a phenomenon probably occurs infrequently; however, it may be considered a means by which monosomic lines might become contaminated.

Since many nullisomics are either male-or female-sterile, and since nullisomics tend to acquire compensating trisomes, nullisomics do not generally form stable lines. Where nullisomics are required they are generally obtained from self-fertilized monosomics.

Although genetic analysis of polyploid wheats is complicated by the fact that there is considerable duplication and triplication of genetic material, the number and allelic relationships of genes controlling many characters have been determined in these wheats

(review by Ausemus, et al., 1946). Since many of the characters studied are di-, tri-, or multigenically controlled, such characters cannot readily be subjected to linkage analysis.

In a study of awnedness in a hexaploid wheat, Stewart (1928) found that the character was digenically controlled, and on the basis of an observed deviation from a 15-to-1 F_2 ratio, calculated a recombination value of 35 percent between the non-allelic genes controlling the character. Briggs (1940) has demonstrated, in a similar manner, a linkage between genes controlling resistance to bunt in a hexaploid wheat variety. Stanford (1941) has demonstrated the linkage of a third gene controlling bunt-resistance to the linkage group established by Briggs.

Linkage between genes controlling different characters has also been established in several instances in the hexaploid wheats. Twenty-eight percent recombination has been calculated between genes controlling awnedness and keeled glumes (Watkins, 1928). Recombination values have also been obtained between genes controlling glume color and gluten strength (Worzella, 1942), leaf rust resistance and mildew resistance genes (Wells and Swenson, 1944), and between several other pairs of alleles (review by Ausemus, et al., 1946).

Although certain attempts at conventional linkage analysis have been successful, many attempts have been inconclusive. Shen, et al., (1938) noted an indication of linkage in the F_2 of a cross

between a beardless, blunt-beak variety and a bearded, pointed-beak variety of hexaploid wheat, although only one of the two expected recombinant classes was present. Similarly, Pan (1940) observed an association between genes controlling resistance to stem rust and susceptibility to black chaff; however, no recombinant class containing individuals susceptible to both diseases was present, although the other recombinant class was present. Other linkage studies with polyploid wheats have been inconclusive (review by Ausemus, et al., 1946), mainly because of a lack of phenotypic segregation.

Linkage analysis, using chromosome deficient aneuploids, has provided an alternative to conventional linkage analysis in polyploids. Clausen and Goodspeed (1928, a, b), as cited by Clausen and Cameron (1944) have reported the use of monosomics to establish a gene-chromosome association in Nicotiana tabacum. Clausen (1941) has described a method of F_1 analysis which has been used to establish a number of gene-chromosome associations in Nicotiana tabacum (Clausen and Cameron, 1944). The method involves crossing monosomic lines of a variety carrying dominant alleles with a variety carrying recessive alleles at the same locus. It is expected that only the critical line will show segregation, since monosomics in only this line should express the recessive phenotype.

F_1 monosomic analysis in hexaploid wheat has been utilized to associate genes controlling awn expression, rust resistance, and dwarfing with specific chromosomes (review by Kuspira and Unrau, 1959).

Subsequently, this method has been used by Tsunewaki and Kihara (1961) to associate a necrosis gene and growth habit genes with specific chromosomes.

Sears (1944) has associated genes controlling seed color, node pubescence, and other characters with specific chromosomes by noting the absence of expression of these characters in particular nullisomics.

A more widely applicable method of aneuploid analysis is the F_2 method briefly described by Sears (1953). Unrau (1950) has used this method to associate genes controlling glume color, growth habit, and other characters with specific chromosomes. Subsequently the F_2 method has been used by a number of workers to establish gene-chromosome associations in the hexaploid wheats and also in Nicotiana tabacum (review by Kuspira and Unrau, 1959). Kuspira and Unrau (1959) have discussed in considerable detail methods of F_2 analysis which might be employed to locate genes showing various kinds of allelic action and gene interactions on specific chromosomes.

Sears (1953) described a method of linkage analysis which utilizes chromosome substitutions. Substitution lines have since been used to associate genes controlling stem rust resistance (Sears, et al., 1957), awning, earliness, and other characters (Kuspira and Unrau, 1957) with specific chromosomes in hexaploid wheats.

Sears (1953) also described a method of F_3 aneuploid analysis. No F_3 analyses have been reported as useful except in confirmation of results obtained from F_2 analysis.

Although a monosomic series in a tetraploid wheat would greatly simplify linkage analysis in these wheats, no such series has been developed. Tsunewaki (1964) has shown that although monosomics can be produced in the tetraploid wheats, the monosomic condition is not transmitted to the offspring due to the low fertility of chromosome-deficient female gametes.

Because duplication of genetic material complicates linkage analysis, and since monosomic series do not exist in tetraploid wheats, alternative methods of linkage analysis are desirable to simplify analysis in these wheats.

2. Genetics of the character studied

Wheats may generally be classified as having spring or winter growth habits. Winter wheats are those wheats which require fall planting in order to mature the following summer. Spring wheats, however, have a shorter vegetative period and consequently will mature during one growing season. Winter wheats generally display a spreading growth habit during the early vegetative period, whereas spring wheats are usually comparatively erect.

Many wheats, referred to as having winter growth habits, will head during a single growing season; however, heading is usually delayed. Because classification with respect to growth habit is somewhat arbitrary, classification for genetic studies is usually based on some particular aspect of growth habit, such as days to heading, spreading versus erect growth habit, winter hardiness, or other characteristics which may be measured or contrasted fairly critically.

A number of workers have found spring growth habit dominant to winter growth habit, and the simple ratios usually obtained suggest monogenic or digenic control of the character (Fruwirth, et al., 1910, as reviewed by Cooper, 1923; Vavilov and Kuznetson, 1921, as reviewed by Cooper, 1923; Cooper, 1923; Aamodt, 1927; Hayes and Aamodt, 1927; Stewart, 1931; and Quisenberry, 1931). Aamodt (1923) and Gaines and Singleton (1926) have found spring growth habit dominant to winter growth habit; however, from the ratios obtained in their crosses they suggest multigenic control of the character. Powers (1934), on the basis of a detailed study of several aspects of growth habit, has suggested trigenic control of the character. He concluded that dominant alleles at two loci and recessive alleles at a third locus result in spring growth habit and that the alleles for spring growth habit are epistatic to those for winter growth habit.

Using monosomic analysis, Unrau (1950) concluded that duplicate genes control growth habit, and he associated one of these genes with chromosome IX of the hexaploid variety Hymar. Morrison (1960), as reviewed by Tsunewaki and Jenkins (1961), has located genes affecting response to vernal treatment on chromosomes IX and XVIII of hexaploid varieties. Tsunewaki and Jenkins (1961) have located growth habit genes on chromosomes IX, XIII, and XVIII of hexaploid varieties. Kuspira and Unrau (1957), using chromosome substitutions have concluded that genes controlling growth habit are carried on chromosomes XIII and XVIII of the variety Thatcher.

The genetics of growth habit has not been studies in the tetraploid wheats. However, since chromosomes IX and XIII are likely both in the A genome, and certainly are either in the A or B genomes, the tetraploid wheats might be expected to carry growth habit genes on these chromosomes. Moreover, since winter growth habit is usually found to be recessive to spring growth habit in the hexaploid wheats, it is likely that the same allelic relationship exists in the tetraploid wheats.

MATERIALS AND METHODS

Although a more detailed outline of the procedure and materials used is presented, the general outline of the procedure is as follows:

1. Crosses were made between a tetraploid winter wheat variety and the first 14 monosomic lines of hexaploid spring wheat varieties.

2. The F_1 generation was analysed both genetically and cytologically.

1. Varieties used

The tetraploid variety used was Triticum durum var. Caid Eleize, grown from local stocks, seed for which was originally obtained from Morocco.

The hexaploid monosomics used in the crosses were monosomic lines I to XIV of Triticum aestivum var. Chinese Spring and Triticum aestivum var. Redman. The Chinese Spring monosomics were grown from local stocks originally obtained from E.R. Sears. The Redman monosomics were grown from local seed stocks originally obtained from R.C. McGinnis.

Both Chinese Spring and Redman headed earlier than Caid Eleize (75 days, 63 days, and 79 days in summer field, 1965). On the basis that the hexaploids headed earlier than the tetraploid ,

the hexaploids and the tetraploid were classified as displaying spring and winter growth habits respectively. This does not imply that these hexaploids and the tetraploid are typically spring and winter types with respect to all other growth habit characteristics.

2. Crossing procedure

In order to equalize heading dates so that crosses could be made, the tetraploid was vernalized as follows: Seeds were germinated in trays containing moist vermiculite at room temperature. When the seedlings reached a height of approximately two inches, the trays, with seedlings, were placed in a dark room at 5^{o} C. and left for 45 days. The seedlings were then exposed to continuous lighting at room temperature for two days. The seedlings were then transplanted in the field at the University farm.

Monosomic lines of the hexaploids used in the crosses were seeded seven days prior to the transplanting of the tetraploid seedlings. Seeds of monosomic lines were planted individually by hand, approximately eight inches apart, in rows separated by one foot.

Since only monosomics were used in crosses, plants of each line were analysed cytologically so that at least three monosomic plants in each line could be selected for crossing. Spikes for pollen mother cell analysis were collected when the flag leaf of a tiller had emerged approximately one inch from the boot. Spikes were fixed in a solution of six parts alcohol, three parts chloroform, and one part acetic acid.

Anthers were stained in aceto carmine and dividing meiocytes observed using a Reichert model 241-427 microscope at a magnification of approximately 360. Slides were made permanent by the quick-freeze technique described by Conger and Fairchild (1953). Plants showing 20 bivalents plus one univalent at metaphase I or 41 chromosomes at anaphase I, in at least three well isolated cells, were classified as being monosomic for the chromosome concerned.

Crosses were made between the tetraploid variety used as a male parent and three different monosomic plants from each of the first 14 monosomic lines of each of Chinese Spring and Redman. Crosses were accomplished as follows:

1. Plants to be used as female parents were emasculated when anthers in the central region of the spike were nearly mature. Emasculation was effected by the removal, with forceps, of all anthers in the spike. Emasculated spikes were immediately bagged.

2. The emasculated spikes were pollinated when stigmas in the florets of central spikelets became enlarged and feathery (two to four days after emasculation).

3. Pollination was effected by bursting mature anthers from the plants used as male parents against the inner surfaces of the lemma and palea of each floret of the measculated spikes.

4. Pollinated spikes were kept bagged until mature.

3. Analysis of F_1's

Twenty F_1 seeds of each line produced from crosses between the Chinese Spring monosomics and Caid Eleize were seeded in the University greenhouse in December, 1964. The seeds were planted 8 inches apart in 3-foot rows which were spaced 10 inches apart. Parental varieties were also seeded in a similar manner.

The F_1 plants and parental varieties were classified with respect to spreading versus erect growth habit and with respect to days taken to head. A plant was considered headed when one or more spikes were completely emerged from the boot.

The remaining seeds from the crosses between Chinese Spring and Caid Eleize, and the seeds from crosses between Redman monosomics and Caid Eleize, as well as the parental varieties were seeded at the University farm in May, 1965. The seeds were space-planted in ten-foot rows and such that plants were at least eight inches apart. The rows were spaced one foot apart.

F_1 plants and parental varieties were classified with respect to spreading versus erect growth habit and with respect to obvious differences in rate of growth. Since only the crosses between Redman monosomics and Caid Eleize showed obvious segregation for slow-versus fast-growing plants, as expressed through the appearance of some comparatively short plants in line IX, only this series of crosses was analysed cytologically and classified with respect to days taken to head. Cytological analysis was made on enough plants

in all 14 lines to determine at least 10 monosomics and 5 disomics in each line. The significance of the difference between the mean heading date of disomics and monosomics in each line was estimated using the "Student" t-test.

Photographs of field plants were taken using an Asahi Pentax, model S1, 35 mm. camera. Photographs of meiotic cells were taken with a Leitz Laborlux 2 microscope under phase contrast using a 6-1/2 x 9 cm. camera.

RESULTS

Crosses between Chinese Spring monosomics and Caid Eleize yielded the following results:

1. Greenhouse, winter, 1964-65

The parental varieties, Chinese Spring and Caid Eleize, as well as all plants in the F_1 of crosses between Chinese Spring monosomics and Caid Eleize, showed an erect growth habit.

Caid Eleize headed later than the Chinese Spring variety (79 and 75 days respectively). The F_1 lines of crosses between the first 14 lines of Chinese Spring and Caid Eleize showed no obvious segregation into early and late heading groups (see appendix, Table IV).

2. Summer, field, 1965

A. Chinese Spring monosomics x Caid Eleize

Neither the parental varieties nor the F_1's of crosses between Chinese Spring monosomics and Caid Eleize showed any obvious segregation for spreading versus erect growth habit when grown in the summer, 1965, at the University farm. There was no obvious segregation for rate of growth in the F_1 of these crosses.

Although the parental varieties, Chinese Spring and Caid Eleize, differed in heading dates (75 and 79 days required to head respectively), since there was no obvious segregation for rate of

growth, as determined by obvious differences in plant height at any time during the growing season, heading dates were not recorded nor was a cytological analysis made of the F_1's of these crosses.

B. Redman monosomics x Caid Eleize

Although no segregation for spreading versus erect growth habit was apparent in any other F_1 line of crosses between Redman monosomics and Caid Eleize, 30 of the 41 F_1 plants in line IX were considerably slower growing, as indicated by plant height after 42 days of growth, than all other plants in the same and other lines (see Figure 1).

Since segregation for growth habit was apparent, a cytological analysis of plants in the F_1 lines was made and heading dates of the parental varieties and F_1 plants were noted (see Table II).

The varieties Redman and Caid Eleize headed 63 and 79 days after seeding respectively.

A t-test designed for the analysis of groups of different sizes (explained by Snedecor, 1961) was used to determine whether or not there was a significant difference between the time required to head by monosomics and the time required to head by disomics in each line. A significant difference was observed in lines V and IX.

The cytological analysis of chromosomes of metaphase I of meiosis in the hybrids showed almost exclusively either 14 bivalents

and 7 univalents or 13 bivalents and 8 univalents (Figs. 2, 3, 4, and 5). Either 34 or 35 chromosomes were observed at anaphase I of meiosis (Figs. 5 and 6). No cells observed showed multivalents at metaphase I. Rarely, a deficit of bivalents and an excess of univalents were observed. Some of these cells might have been the result of early separation of bivalents rather than a failure of chromosomes to synapse.

Thompson (1927) described the meiosis of pentaploid wheat hybrids and concluded that all the univalents divide equationally at the first division. It was obvious from my work that at least some of the univalents divide equationally at the first division (Figure 7). However, it is not possible to conclude that all univalents divide in this manner during meiosis, since studies of the second division were not undertaken.

Table II. Differences in days to heading between monosomics and disomics in F_1 lines of crosses between Redman monosomics and Caid Eleize.

F_1 line	Monosomics		Disomics		Delayed heading of monosomics in days
	No. of plants	Average days to heading	No. of plants	Average days to heading	
I	9	72.1	6	72.3	-0.2
II	10	71.8	6	69.2	2.6
III	12	70.8	6	70.5	0.3
IV	11	71.0	5	72.4	-1.4
V	13	72.2	7	69.4	2.8*
VI	10	71.8	6	70.5	1.3
VII	11	71.9	6	71.5	0.4
VIII	11	72.9	7	71.0	1.9
IX	12	80.9	7	69.3	11.6**
X	11	68.6	6	69.5	-0.9
XI	14	70.6	6	69.8	0.8
XII	9	73.4	5	72.6	0.8
XIII	10	71.2	5	72.0	-0.8
XIV	11	66.8	5	69.4	-2.6
Redman disomics x Caid Eleize			17	70.6	

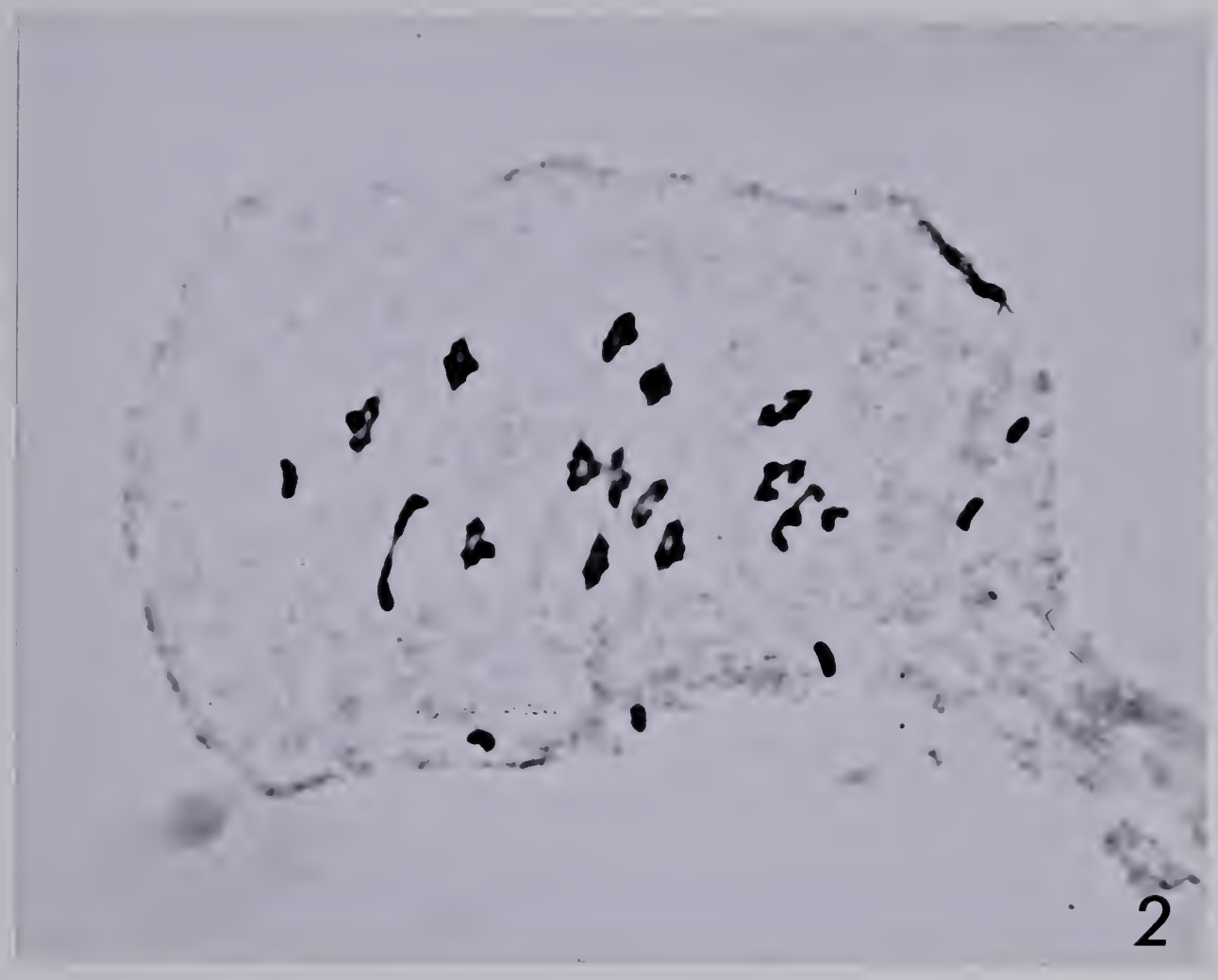
* Significant at the 5% level using the t-test.

** Significant at the 1% level using the t-test.

Figure 1. Photograph of F_1 plants in line IX of a cross between Redman monosomics and Caid Eleize (after 42 days of growth).

Figure 2. Photomicrograph of a meiotic metaphase I of an F_1 hybrid of a cross between Redman monosomic VII and Caid Eleize, showing 14 bivalents and 7 univalents. Magnification approx. 1030.

Figure 3. Photomicrograph of a meiotic metaphase I of an F_1 hybrid of a cross between Redman monosomic III and Caid Eleize, showing 14 bivalents and 7 univalents. Magnification approx. 1030.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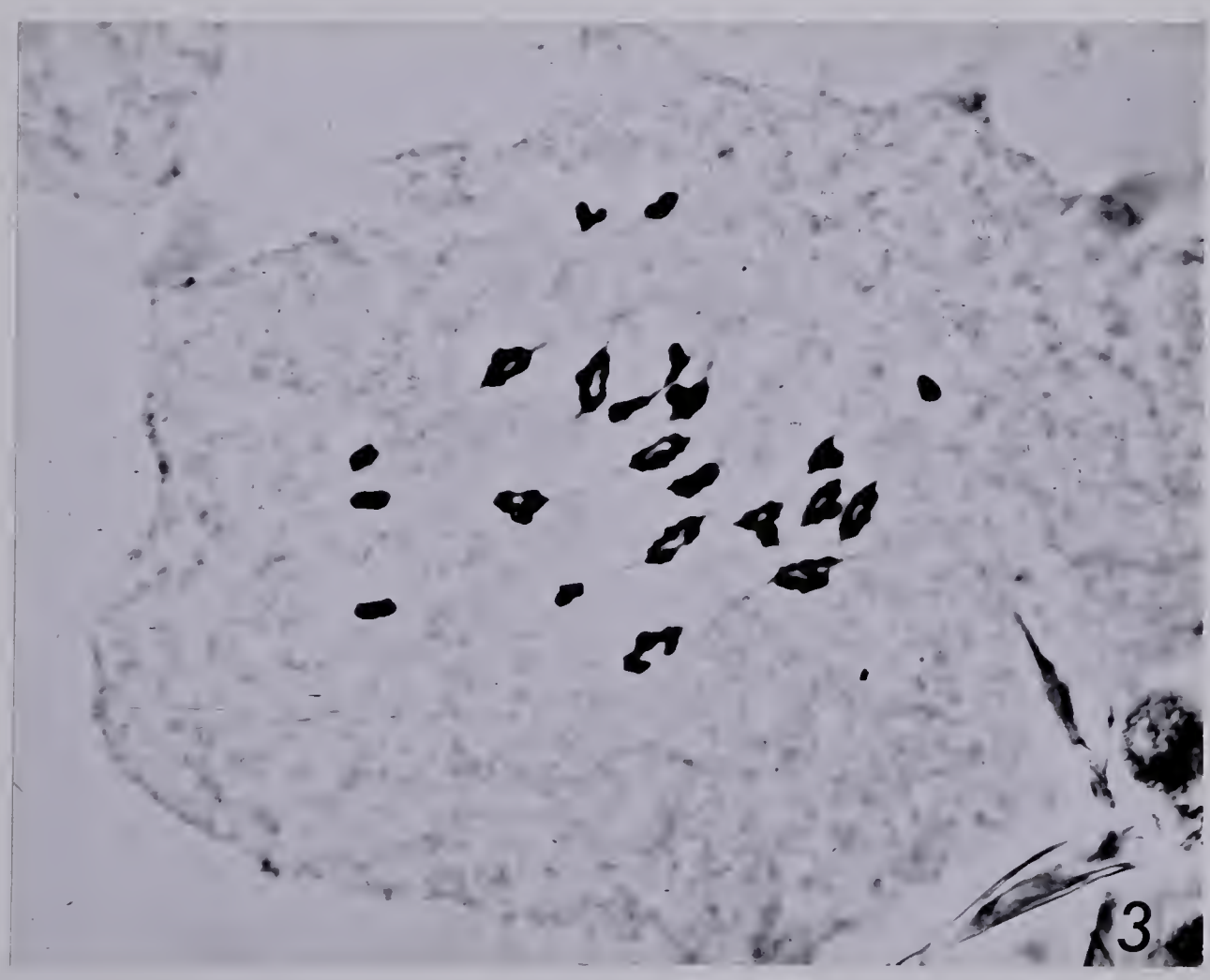
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Figure 4. Photomicrograph of a meiotic metaphase I of an F_1 hybrid of a cross between Redman monosomic IV and Caid Eleize, showing 13 bivalents and 8 univalents. Magnification approx. 1030.

Figure 5. Photomicrograph of a meiotic metaphase I of an F_1 hybrid of a cross between Redman monosomic VII and Caid Eleize, showing 13 bivalents and 8 univalents. Magnification approx. 1030.

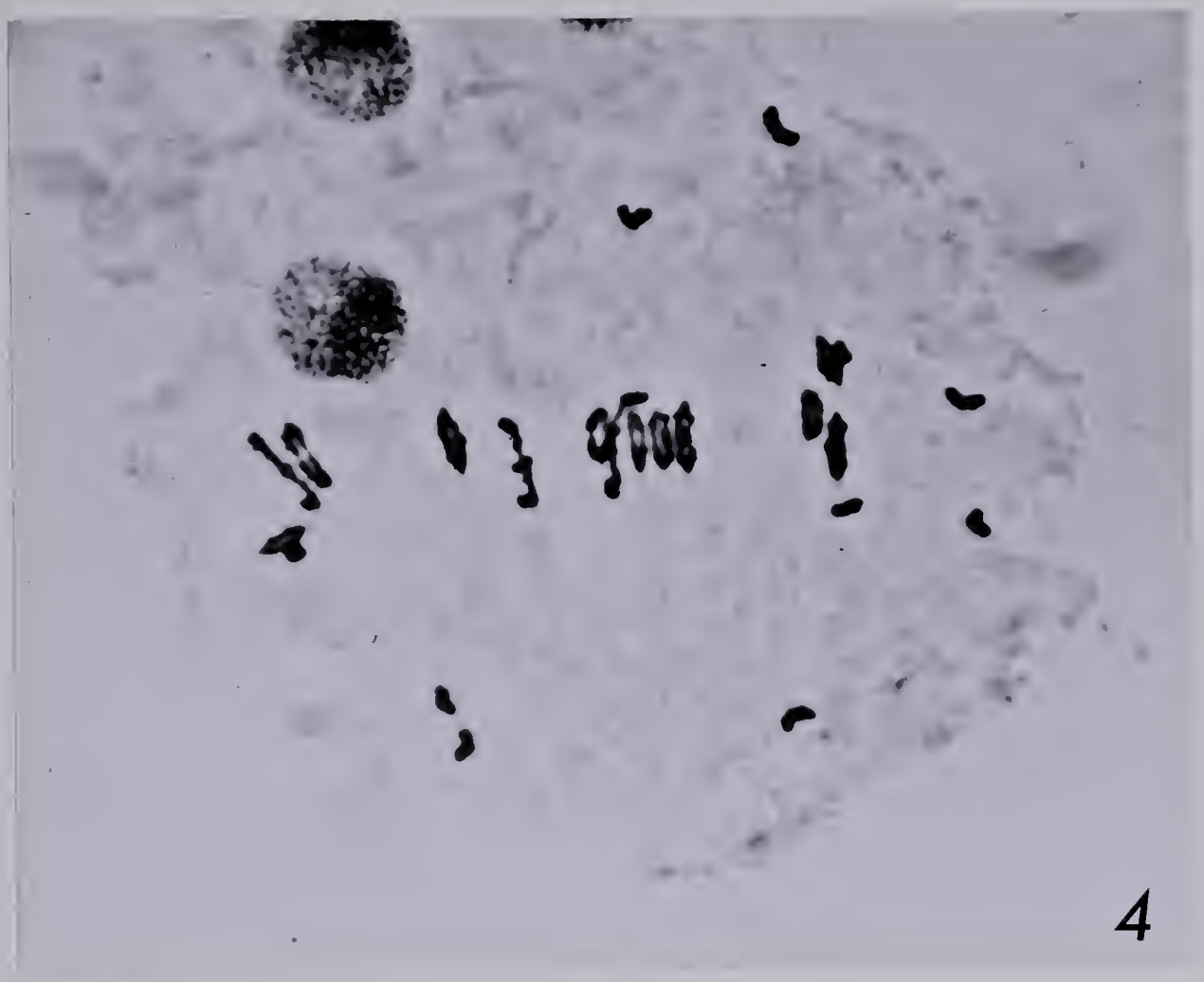

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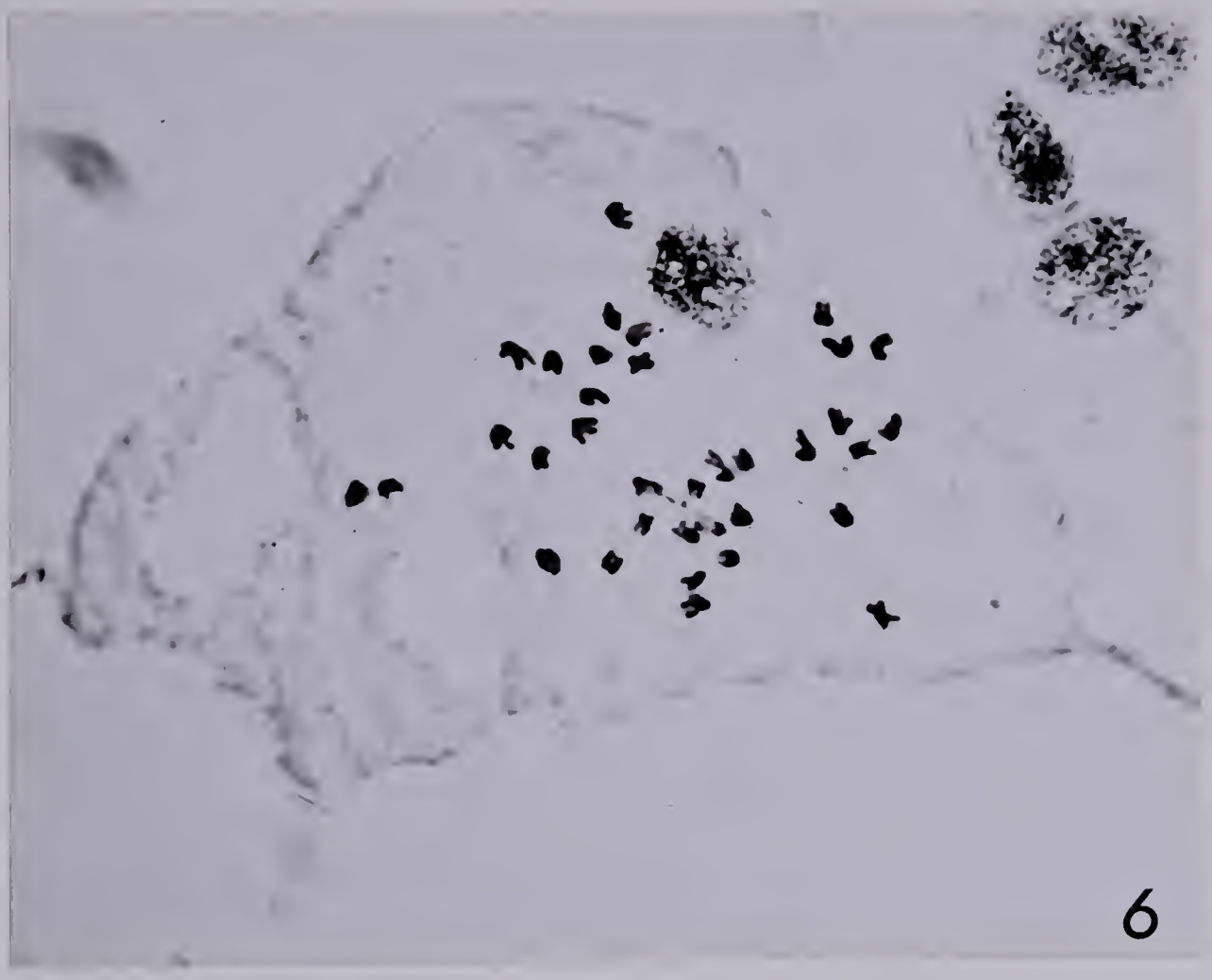
Figure 6. Photomicrograph of a meiotic anaphase I of an F_1 hybrid of a cross between Redman monosomic VI and Caid Eleize, showing 35 chromosomes.
Magnification approx. 1030.

Figure 7. Photomicrograph of a meiotic anaphase I of an F_1 hybrid of a cross between Redman monosomic V and Caid Eleize, showing 13 chromosomes at each pole and 8 univalents near the metaphase plate , some of which are dividing equationally.
Magnification approx. 1030.



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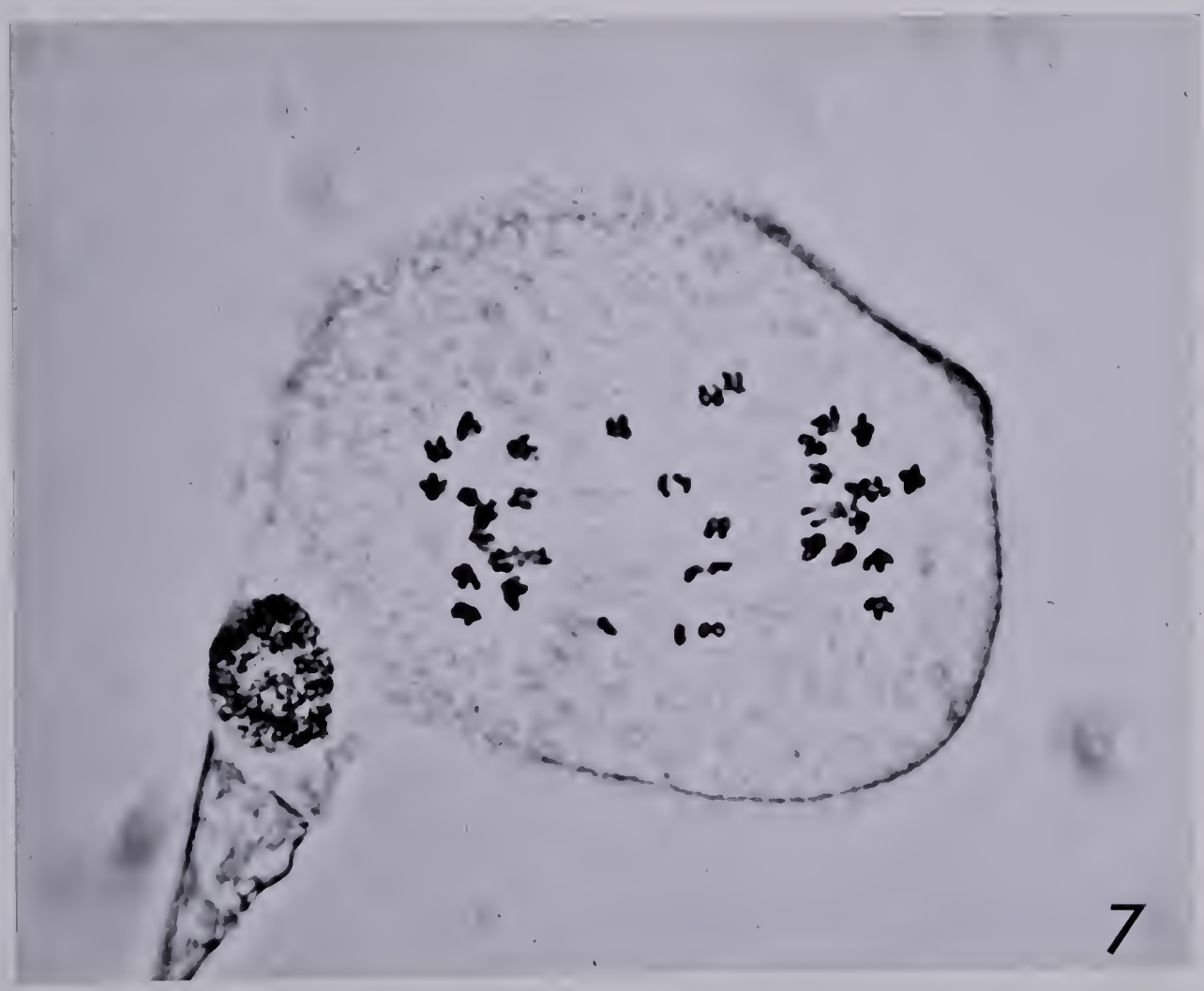

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DISCUSSION

Theoretically two methods might be employed to establish gene-chromosome associations in tetraploid wheat using common wheat aneuploids. One method is to produce a triploid by crossing a tetraploid variety with Aegilops squarrosa (D genome), polyploidize the hybrid, and analyse the F_1, F_2, and F_3 generations of crosses between the artificially produced hexaploid and a series of hexaploid monosomics or nullisomics. Analysis could be made using ratios and tables proposed for analysis of hexaploids (Kuspira and Unrau, 1959).

An alternative method would be to cross the tetraploid with the first 14 lines of the hexaploid monosomics or nullisomics and analyse the F_1 generation genetically or cytogenetically. If tetraploids are crossed with hexaploid monosomics, the pentaploid hybrids will all be monosomic for chromosomes XV to XXI, as well as being either monosomic or disomic for one of the first 14 chromosomes.

Chromosomes of tetraploid wheats carrying particular genes can theoretically be detected by F_1 analysis of crosses between hexaploid monosomics and tetraploids, if the chromosomes concerned carry incompletely dominant or recessive alleles. Chromosomes carrying hemizygous ineffective recessives or recessives whose expression is obscured by dominant alleles at other loci cannot be identified by this method. Table III illustrates how analysis of

the F_1 of crosses between hexaploid monosomics and tetraploids can be utilized to associate a gene with a specific chromosomes.

Table III. Theoretical table illustrating the analysis of F_1 lines of crosses between hexaploid monosomics, carrying a dominant allele (A) on one of chromosomes I to XIV, and a tetraploid carrying recessive alleles (aa).

"Critical" F_1's		"Non-critical" F_1's		Normal F_1's	
♀ gamete \ ♂ gamete	$13^I + 1^I(a)$	♀ gamete \ ♂ gamete	$13^I(a) + 1^I$	♀ gamete \ ♂ gamete	$14^I(a)$
$13^I+1^I(A)+7^I$	Disomic* (25%) $13^{II}+1^{II}(Aa)+7^I$ (phenotypically dominant)	$13^I(A)+1^I+7^I$	Disomic* (25%) $13^{II}(Aa)+1^{II}+7^I$ (phenotypically dominant)	14^I+7^I	Disomic* $14^{II}(Aa)+7^I$ (phenotypically dominant)
$13^I + 7^I$	Monosomic**(75%) $13^{II}+1^I(a)+7^I$ (phenotypically recessive)	$13^I(A) + 7^I$	Monosomic** (75%) $13^{II}(Aa)+1^I+7^I$ (phenotypically dominant)		

* Disomic for chromosomes I to XIV.

** Monosomic for one of chromosomes I to XIV.

Monosomic analysis to determine the location of a recessive allele depends on the assumption that the absence of expression of a dominant allele due to the absence of a particular chromosome indicates the presence of a recessive allele on the homologous chromosome. In interspecific hybrids true homology may or may not exist between chromosomes of the species concerned.

The absence of bivalent pairing, expressed either as a high degree of asynapsis or by the presence of multivalents during meiosis of the hybrid between two species, would indicate that the chromosomes of the two species are not completely homologous. In polyploid wheats, however, bivalent pairing has been shown to be under genetic control (Riley, 1960); thus the absence of bivalent pairing, particularly in aneuploids, may not necessarily indicate a high degree of non-homology between the chromosomes of the two species.

The F_1 hybrids produced from crosses between Redman and Caid Eleize consistently showed 14 bivalents and 7 univalents or 13 bivalents and 8 univalents at metaphase I of meiosis (Figures 2, 3, 4, and 5), indicating that a high degree of homology likely exists between the chromosomes of Caid Eleize and chromosomes I to XIV of Redman.

One of the difficulties of a cytogenetic analysis of tetraploids by crossing tetraploids with hexaploid monosomics is that the F_1 of such crosses are pentaploid, and as such are fairly sterile. Moreover, such hybrids will not breed true for the original chromosome deficiencies. Thus neither the genetic nor the cytogenetic analysis can readily be carried beyond the F_1 generation.

The F_1 is sufficient to provide some information concerning allelic relationships and to establish gene-chromosome associations, although its applicability is restricted to certain kinds of allelic action. The method of analysis using artificially produced hexaploids

would have the advantage of allowing analysis beyond the F_1 generation. Genes present in the D genome might, however, obscure segregation of genes in the A and B genomes.

Since Caid Eleize headed relatively late (79 days) and Redman headed relatively early (63 days), and the disomic F_1's headed fairly early (70.6 days), late heading is not completely dominant to early heading in these varieties. The monosomics in line IX headed considerably later than their disomic sibs (80.9 and 69.3 days respectively). The monosomics in line V headed slightly later than their disomic sibs (72.2 and 69.4 days respectively).

The segregation for growth habit in line IX, expressed as a segregation of tall (disomic) and short (monosomic) plants after 42 days of growth, and as a segregation of early (disomic) and late heading (monosomic) plants indicates that chromosome IX of Caid Eleize carries recessive or incompletely dominant alleles affecting growth habit. Although the difference in days to heading of monosomics and disomics in line V was significant according to the t-test, the difference (2.8 days) was not great. Thus only with much less certainty than in the case of chromosome IX can we say that chromosome V of Caid Eleize carries a gene or genes affecting growth habit.

Although the disomic F_1's showed somewhat of an intermediate phenotype with respect to time required to head, as compared to the parental varieties, because the F_1 plants were all monosomic for

chromosomes XIV to XXI, the intermediate phenotype might be due to genes other than the ones located on chromosomes V and IX. Thus it is not possible in this case to determine whether the alleles affecting growth habit in the variety Caid Eleize are recessive or incompletely dominant.

The absence of obvious segregation in the crosses between Chinese Spring monosomics and Caid Eleize might be due in part to the lack of sufficient genetic difference, with respect to growth habit, between these two species. The comparatively small difference in days to heading in these species (4 days) might be due to the specific environments under which the plants were grown even though specific genetic differences exist. However, since there was no obvious segregation either under greenhouse conditions or under field conditions, genetic differences with respect to growth habit in these species is probably slight. If a cytological analysis had been made, perhaps segregation might have been detected in this series of crosses.

Cytological analysis of the F_1 generation may not be necessary to establish gene-chromosome associations. The gene or genes located on chromosome IX for example could have been associated with this chromosome because of the obvious segregation for growth habit in line IX of the crosses. Less obvious segregation such as that detected in line V might not, however, have been detected in the absence of a cytological analysis. A cytological examination of some F_1 plants is advisable so that one can assess the extent of homology between the chromosomes of the species concerned.

From the results of this study, it seems reasonable that the location of other genes with non-dominant alleles might be determined in tetraploid wheats by the method used in this study.

Knowledge of gene-chromosome associations in the tetraploid wheats may be helpful in assessing the extent of homology between the genomes of different species. Knowledge of gene-chromosome associations in tetraploid wheats may also be of use to plant breeders who wish to transfer particular characters from the tetraploid wheats to the hexaploid wheats by chromosome substitutions.

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APPENDIX

Table IV. Days to heading of 20 F_1 hybrids from each line of crosses between Chinese Spring monosomic lines I to XIV and Caid Eleize. Grown in greenhouse, University of Alberta, Edmonton. Seeded December, 1965.

Line	Days to heading																			
I	70	72	71	67	67	71	70	72	70	71	67	71	71	71	79	72	*	67	67	72
II	74	70	68	71	73	*	68	72	73	71	73	70	71	71	71	76	74	72	77	71
III	70	70	70	67	68	75	72	71	71	73	73	71	73	75	72	70	73	70	71	71
IV	73	73	67	71	68	74	*	73	76	73	71	72	70	71	71	73	74	73	70	75
V	74	80	72	71	70	73	73	78	76	73	72	72	83	74	78	78	80	74	74	78
VI	83	78	76	71	*	71	71	72	72	72	*	73	72	74	74	75	71	67	68	71
VII	*	78	78	73	76	75	72	78	76	76	78	76	74	78	78	78	78	78	74	74
VIII	73	*	*	73	73	71	71	70	*	70	72	70	73	78	77	77	*	73	*	73
IX	75	72	73	74	*	76	78	80	73	73	70	71	74	80	76	*	76	73	73	78
X	75	72	70	72	73	70	78	75	72	70	70	*	72	72	74	*	80	73	78	*
XI	76	76	78	73	80	78	75	68	70	71	71	70	*	72	74	70	72	70	71	71
XII	72	*	72	73	71	72	72	68	68	68	68	70	71	70	70	70	72	70	*	72
XIII	74	70	72	75	71	75	*	78	72	73	78	*	75	*	70	72	75	83	78	73
XIV	70	70	67	75	70	70	70	70	*	70	*	72	71	*	70	*	72	73	78	75
Chinese Spring x Caid Eleize	78	75	75	75	78	78	78	76	78	76	77	77								

* Did not germinate.